

SEQUENCE LISTING



<110> Leo G.J. FRENKEN
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Vin-Miin TEH
Martine E. VERHOEYEN
Joy E. WILKINSON
Stephen A. JOBLING

<20> Production of Antibodies

<130> PNK/060113/0275850 - T7060C

<140> US 09/737,476

<141> 2000-12-18

<150> EP 99310188.0

<151> 1999-12-17

<160> 67

<170> MS Word

<210> 1

<211> 440

<212> DNA

<213> Artificial Sequence

<220>

<223> VHH with peptide linker

<220>

<221> CDS

<222> (1)..(417)

<400> 1

cag	gtg	cag	ctg	cag	gag	tca	ggg	gga	gga	ttg	gtg	cag	gct	ggg	ggc	48
Gln	Val	Gln	Leu	Gln	Glu	Ser	Gly	Gly	Gly	Leu	Val	Gln	Ala	Gly	Gly	
1			5						10					15		

tct	ctg	aga	ctc	tcc	tgt	gca	gcc	tcg	gga	cgc	gcc	acc	agt	ggt	cat	96
Ser	Leu	Arg	Leu	Ser	Cys	Ala	Ala	Ser	Gly	Arg	Ala	Thr	Ser	Gly	His	
		20						25					30			

ggt	cac	tat	ggt	atg	ggc	tgg	ttc	cgc	cag	gtt	cca	ggg	aag	gag	cgt	144
Gly	His	Tyr	Gly	Met	Gly	Trp	Phe	Arg	Gln	Val	Pro	Gly	Lys	Glu	Arg	
		35				40						45				

gag	ttt	gtc	gca	gct	att	agg	tgg	agt	ggt	aaa	gag	aca	tgg	tat	aaa	192
Glu	Phe	Val	Ala	Ala	Ile	Arg	Trp	Ser	Gly	Lys	Glu	Thr	Trp	Tyr	Lys	
	50					55					60					

gac tcc gtg aag ggc cga ttc acc atc tcc aga gat aac gcc aag act 240
Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Thr
65 70 75 80

acg gtt tat ctg caa atg aac agc ctg aaa cct gaa gat acg gcc gtt 288
Thr Val Tyr Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Val
85 90 95

tat tat tgt gcc gct cga ccg gtc cgc gtg gat gat att tcc ctg ccg 336
Tyr Tyr Cys Ala Ala Arg Pro Val Arg Val Asp Asp Ile Ser Leu Pro
100 105 110

gtt ggg ttt gac tac tgg ggc cag ggg acc cag gtc acc gtc tcc tca 384
Val Gly Phe Asp Tyr Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
115 120 125

gaa caa aaa ctc atc tca gaa gag gat ctg aat taataagggc taagctcgaa 437
Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu Asn
130 135

ttc 440

<210> 2

<211> 139

<212> PRT

<213> Artificial Sequence

<220>

<223> VHH with peptide linker

<400> 2

Gln Val Gln Leu Gln Glu Ser Gly Gly Gly Leu Val Gln Ala Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Arg Ala Thr Ser Gly His
20 25 30

Gly His Tyr Gly Met Gly Trp Phe Arg Gln Val Pro Gly Lys Glu Arg
35 40 45

Glu Phe Val Ala Ala Ile Arg Trp Ser Gly Lys Glu Thr Trp Tyr Lys
50 55 60

Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Thr
65 70 75 80

Thr Val Tyr Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Val
85 90 95

Tyr Tyr Cys Ala Ala Arg Pro Val Arg Val Asp Asp Ile Ser Leu Pro
100 105 110

Val Gly Phe Asp Tyr Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
115 120 125

Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu Asn
 130 135

<210> 3

<211> 11

<212> PRT

<213> Artificial Sequence

<220>

<223> myc linker

<400> 3

Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu Asn
 1 5 10

<210> 4

<211> 471

<212> DNA

<213> Artificial Sequence

<220>

<223> VHH with linker

<220>

<221> CDS

<222> (1)..(459)

<400> 4

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 Gln Val Gln Leu Gln Gln Ser Gly Gly Gly Leu Val Gln Ala Gly Gly
 1 5 10 15

tct ctg aga ctc tcc tgt gta gct tct gaa agc agc ttc agc aac aat 96
 Ser Leu Arg Leu Ser Cys Val Ala Ser Glu Ser Ser Phe Ser Asn Asn
 20 25 30

cac atg ggc tgg tac cgc cgg gct cca ggg aac cag cgc gag ctg gtc 144
 His Met Gly Trp Tyr Arg Arg Ala Pro Gly Asn Gln Arg Glu Leu Val
 35 40 45

gca act att agt cct ggt ggt agc aca cac tat gta gac tcc gtg aag 192
 Ala Thr Ile Ser Pro Gly Gly Ser Thr His Tyr Val Asp Ser Val Lys
 50 55 60

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ggc cga ttc acc atc tcc cga gac aac gcc aag aac aca gtg tat cta 240
Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Val Tyr Leu
65 70 75 80

caa atg gac agc ctg aaa cca gag gac acg gcc gtc tat tac tgt gct 288
Gln Met Asp Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys Ala
85 90 95

gcc aag ggg agg ggg ctg cag gct atg cag tac tgg gcc cag ggg acc 336
Ala Lys Gly Arg Gly Leu Gln Ala Met Gln Tyr Trp Gly Gln Gly Thr
100 105 110

ctg gtc acc gtc tcc tca gcg cac cac agc gaa gac ccc agc tcc gcg 384
Leu Val Thr Val Ser Ser Ala His His Ser Glu Asp Pro Ser Ser Ala
115 120 125

gcc gcc cat cac cat cac cat cac ggg gcc gca gaa caa aaa ctc atc 432
Ala Ala His His His His His His Gly Ala Ala Glu Gln Lys Leu Ile
130 135 140

tca gaa gag gat ctg aat ggg gcc gca tagtaacaat tg 471
Ser Glu Glu Asp Leu Asn Gly Ala Ala
145 150

<210> 5

<211> 153

<212> PRT

<213> Artificial Sequence

<220>

<223> VHH with linker

<400> 5

Gln Val Gln Leu Gln Gln Ser Gly Gly Gly Leu Val Gln Ala Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Val Ala Ser Glu Ser Ser Phe Ser Asn Asn
20 25 30

His Met Gly Trp Tyr Arg Arg Ala Pro Gly Asn Gln Arg Glu Leu Val
35 40 45

Ala Thr Ile Ser Pro Gly Gly Ser Thr His Tyr Val Asp Ser Val Lys
50 55 60

Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Val Tyr Leu
65 70 75 80

Gln Met Asp Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys Ala
85 90 95

Ala Lys Gly Arg Gly Leu Gln Ala Met Gln Tyr Trp Gly Gln Gly Thr
100 105 110

Leu Val Thr Val Ser Ser Ala His His Ser Glu Asp Pro Ser Ser Ala
115 120 125

Ala Ala His His His His His His Gly Ala Ala Glu Gln Lys Leu Ile
130 135 140

Ser Glu Glu Asp Leu Asn Gly Ala Ala
145 150

<210> 6

<211> 468

<212> DNA

<213> Artificial Sequence

<220>

<223> VHH with linker

<220>

<221> CDS

<222> (1)..(456)

<400> 6

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Gln Val Gln Leu Gln Glu Ser Gly Gly Leu Val Gln Ala Gly Gly
1 5 10 15

tct ctg aga ctc tcc tgt gta gcc tct gga aac acc ttc agt atc ata 96
Ser Leu Arg Leu Ser Cys Val Ala Ser Gly Asn Thr Phe Ser Ile Ile
20 25 30

gct atg gcc tgg tac cgc cag gct cca ggg aag cag cgc gag gtg gtc 144
Ala Met Ala Trp Tyr Arg Gln Ala Pro Gly Lys Gln Arg Glu Val Val
35 40 45

gca agt att aat agt att ggc agc aca aat tat gca gac tcc gtg aag 192
Ala Ser Ile Asn Ser Ile Gly Ser Thr Asn Tyr Ala Asp Ser Val Lys
50 55 60

ggg cga ttc acc atc tcc aga gac aac gcc aag aac aca gtg tat ctg 240
Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Val Tyr Leu
65 70 75 80

caa atg agc agc ctg aaa cct gag gac acg gcc gtc tat tac tgt gct 288
Gln Met Ser Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys Ala
85 90 95

gcc ggt aat ttg ctg gtt aag agg cct tac tgg ggc cag ggg acc ctg 336
Ala Gly Asn Leu Leu Val Lys Arg Pro Tyr Trp Gly Gln Gly Thr Leu
100 105 110

gtc acc gtc tcc tca gaa ccc aag aca cca aaa cca caa cca gcg gcc 384
Val Thr Val Ser Ser Glu Pro Lys Thr Pro Lys Pro Gln Pro Ala Ala
115 120 125

gcc cat cac cat cac cat cac ggg gcc gca gaa caa aaa ctc atc tca 432
Ala His His His His His His Gly Ala Ala Glu Gln Lys Leu Ile Ser
130 135 140

gaa gag gat ctg aat ggg gcc gca tagtaacaat tg 468
Glu Glu Asp Leu Asn Gly Ala Ala
145 150

<210> 7

<211> 152

<212> PRT

<213> Artificial Sequence

 $\langle 220 \rangle$

<223> VHH with linker

<400> 7

Gln Val Gln Leu Gln Glu Ser Gly Gly Gly Leu Val Gln Ala Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Val Ala Ser Gly Asn Thr Phe Ser Ile Ile
20 25 30

Ala Met Ala Trp Tyr Arg Gln Ala Pro Gly Lys Gln Arg Glu Val Val
35 40 45

Ala Ser Ile Asn Ser Ile Gly Ser Thr Asn Tyr Ala Asp Ser Val Lys
50 55 60

Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Val Tyr Leu
65 70 75 80

Gln Met Ser Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys Ala
85 90 95

Ala Gly Asn Leu Leu Val Lys Arg Pro Tyr Trp Gly Gln Gly Thr Leu
100 105 110

Val Thr Val Ser Ser Glu Pro Lys Thr Pro Lys Pro Gln Pro Ala Ala
115 120 125

Ala His His His His His His Gly Ala Ala Glu Gln Lys Leu Ile Ser
130 135 140

Glu Glu Asp Leu Asn Gly Ala Ala
145 150

<210> 8

<211> 462

<212> DNA

037346"9242E260

<213> Artificial Sequence

<220>

<223> VHH with linker

<220>

<221> CDS

<222> (1)..(450)

<400> 8

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Thr Met Ala Gln Val Lys Leu Gln Gln Ser Gly Gly Gly Leu Val Gln	
1 5 10 15	

gct ggg ggc cct ctg agg ctc tcc tgt gca gcc tct gga cgc acc ttc	96
Ala Gly Gly Pro Leu Arg Leu Ser Cys Ala Ala Ser Gly Arg Thr Phe	
20 25 30	

agt aac tat gcc gtg ggc tgg ttc cgc cag gct cca ggg aag gag cgt	144
Ser Asn Tyr Ala Val Gly Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg	
35 40 45	

gag ttt gtc gct gct att agc cgt gat ggt ggg cgc aca tac tat gcg	192
Glu Phe Val Ala Ala Ile Ser Arg Asp Gly Gly Arg Thr Tyr Tyr Ala	
50 55 60	

gac tcc gtg aag ggc cga ttc gcc gtc tcc aga gac tac gcc gag aac	240
Asp Ser Val Lys Gly Arg Phe Ala Val Ser Arg Asp Tyr Ala Glu Asn	
65 70 75 80	

acg gtg tat ctg caa atg aac agc ctg aaa cct gag gac acg gcc gtt	288
Thr Val Tyr Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Val	
85 90 95	

tat tac tgt aac aca agg gcc tac tgg ggc cag ggg acc cag gtc acc	336
Tyr Tyr Cys Asn Thr Arg Ala Tyr Trp Gly Gln Gly Thr Gln Val Thr	
100 105 110	

gtc tcc tca gcg cac cac agc gaa gac ccc agc tcc gcg gcc gcc cat	384
Val Ser Ser Ala His His Ser Glu Asp Pro Ser Ser Ala Ala Ala His	
115 120 125	

cac cat cac cat cac ggg gcc gca gaa caa aaa ctc atc tca gaa gag	432
His His His His Gly Ala Ala Glu Gln Lys Leu Ile Ser Glu Glu	
130 135 140	

gat ctg aat ggg gcc gca tagtaacaat tg	462
Asp Leu Asn Gly Ala Ala	
145 150	

<210> 9

<211> 150

<212> PRT

0934260

Ser Asn Tyr Ala Val Gly Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg
35 40 45
Glu Phe Val Ala Ala Ile Ser Arg Asp Gly Gly Arg Thr Tyr Tyr Ala
50 55 60
Asp Ser Val Lys Gly Arg Phe Ala Val Ser Arg Asp Tyr Ala Glu Asn
65 70 75 80
Thr Val Tyr Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Val
85 90 95
Tyr Tyr Cys Asn Thr Arg Ala Tyr Trp Gly Gln Gly Thr Gln Val Thr
100 105 110
Val Ser Ser Ala His His Ser Glu Asp Pro Ser Ser Ala Ala Ala His
115 120 125
His His His His His Gly Ala Ala Glu Gln Lys Leu Ile Ser Glu Glu
130 135 140
Asp Leu Asn Ser Glu Lys Asp Glu Leu
145 150

<210> 12

<211> 38

<212> DNA

<213> Artificial Sequence

<220>

<223> synthetic insert

<400> 12

agctgcatc gcaagcttgg taccgggaat tctctaga

38

<210> 13

<211> 42

<212> DNA

<213> Artificial Sequence

<220>

<223> synthetic insert

<400> 13

aatttctaga gaattcccg taccaagctt gcttgcgatc gc

42

<210> 14

<400> 17
gaattcccat ggtttacact cgaggtcctc tccaaatga 39

<210> 18
<211> 189
<212> DNA
<213> Artificial Sequence
<220>
<223> PCR product

<400> 18
ccaccacga gggaacatcg tggaaaaaga agacgttoca accacgtctt caaagcaagt 60
ggattgatgt gatatctcca ctgacgtaag ggatgacgca caatcccact atccttcgca 120
agacccttcc tttatataag gaagttcatt tcatttggag aggacctga gtgtaaacca 180
tgggaattc 189

<210> 19
<211> 21
<212> DNA
<213> Artificial Sequence
<220>
<223> sequencing primer

<400> 19
ccggcaacag gattcaatct t 21

<210> 20
<211> 40
<212> DNA
<213> Artificial Sequence
<220>
<223> synthetic insert

<400> 20
agctccatgg gatttggtct cttttcacia ttgccttcat 40

aattaaccct cactaaagg

19

<210> 34

<211> 254

<212> DNA

<213> Artificial Sequence

<220>

<223> GBSS leader

<220>

<221> CDS

<222> (3)..(254)

<400> 34

cc atg gca agc atc aca gct tca cac cac ttt gtg tca aga agc caa	47
Met Ala Ser Ile Thr Ala Ser His His Phe Val Ser Arg Ser Gln	
1 5 10 15	

act tca cta gac acc aaa tca acc ttg tca cag ata gga ctc agg aac	95
Thr Ser Leu Asp Thr Lys Ser Thr Leu Ser Gln Ile Gly Leu Arg Asn	
20 25 30	

cat act ctg act cac aat ggt tta agg gct gtt aac aaa ctt gat ggg	143
His Thr Leu Thr His Asn Gly Leu Arg Ala Val Asn Lys Leu Asp Gly	
35 40 45	

ctc caa tca aga act aat act aag gta aca ccc aag atg gca tcc aga	191
Leu Gln Ser Arg Thr Asn Thr Lys Val Thr Pro Lys Met Ala Ser Arg	
50 55 60	

act gag acc aag aga cct gga tgc tca gct acc att gtt tgt gga aaa	239
Thr Glu Thr Lys Arg Pro Gly Cys Ser Ala Thr Ile Val Cys Gly Lys	
65 70 75	

cag gtg cag ctg cag	254
Gln Val Gln Leu Gln	
80	

<210> 35

<211> 84

<212> PRT

<220>

<223> GBSS leader

<213> Artificial Sequence

<400> 35

Met Ala Ser Ile Thr Ala Ser His His Phe Val Ser Arg Ser Gln Thr
 1 5 10 15
 Ser Leu Asp Thr Lys Ser Thr Leu Ser Gln Ile Gly Leu Arg Asn His
 20 25 30
 Thr Leu Thr His Asn Gly Leu Arg Ala Val Asn Lys Leu Asp Gly Leu
 35 40 45
 Gln Ser Arg Thr Asn Thr Lys Val Thr Pro Lys Met Ala Ser Arg Thr
 50 55 60
 Glu Thr Lys Arg Pro Gly Cys Ser Ala Thr Ile Val Cys Gly Lys Gln
 65 70 75 80
 Val Gln Leu Gln

<210> 36

<211> 18

<212> DNA

<213> Artificial Sequence

<220>

<223> synthetic insert

<400> 36

catgcaggtg cagctgca

18

<210> 37

<211> 10

<212> DNA

<213> Artificial Sequence

<220>

<223> synthetic insert

<400> 37

gctgcacctg

10

<210> 38

<211> 24

<212> DNA

924260

<213> Artificial Sequence

<220>

<223> PCR primer

<400> 38

cgcaagaccc ttctctata taag

24

<210> 39

<211> 60

<212> DNA

<213> Artificial Sequence

<220>

<223> PCR primer

<400> 39

gagctcgaat tcttattata gtcacatcttt ctctgaattc agatcctctt ctgagatgag

60

<210> 40

<211> 26

<212> PRT

<213> Artificial Sequence

<220>

<223> linker

<400> 40

Thr Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
1 5 10 15

Gly Gly Gly Gly Ser Asp Ile Glu Leu Thr
20 25

<210> 41

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> PCR primer

<400> 41

atcctcaact tccaatcaga

20

<210> 42

<211> 19

<212> DNA

<213> Artificial Sequence

<220>

<223> PCR primer

<400> 42

ttcttgagag atagcttga

19

<210> 43

<211> 30

<212> DNA

<213> Artificial Sequence

<220>

<223> synthetic insert

<400> 43

gatcccatgg cccgctagcc aattggagct

30

<210> 44

<211> 22

<212> DNA

<213> Artificial Sequence

<220>

<223> synthetic insert

<400> 44

ccaattggct agcgggccat gg

22

<210> 45

<211> 29

<212> DNA

<210> 49

<211> 25

<212> DNA

<213> Artificial Sequence

<220>

<223> sequencing primer

<400> 49

gtctgtctaa agtaaagtag atgcg

25

<210> 50

<211> 60

<212> DNA

<213> Artificial Sequence

<220>

<223> PCR primer

<400> 50

tccaaccaat tgttatcata gtcactcttt ctactattc agatcctctt ctgagatgag

60

<210> 51

<211> 29

<212> DNA

<213> Artificial Sequence

<220>

<223> PCR primer

<400> 51

agtcccccat ggtacgtcct gtagaaacc

29

<210> 52

<211> 25

<212> DNA

<213> Artificial Sequence

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tat	aaa	gac	tcc	gtg	aag	ggc	cga	ttc	acc	atc	tcc	aga	gat	aac	gcc	239
Tyr	Lys 65	Asp	Ser	Val	Lys	Gly 70	Arg	Phe	Thr	Ile	Ser 75	Arg	Asp	Asn	Ala	
aag	act	acg	gtt	tat	ctg	caa	atg	aac	agc	ctg	aaa	cct	gaa	gat	acg	287
Lys 80	Thr	Thr	Val	Tyr	Leu 85	Gln	Met	Asn	Ser	Leu 90	Lys	Pro	Glu	Asp	Thr 95	
gcc	gtt	tat	tat	tgt	gcc	gct	cga	ccg	gtc	cgc	gtg	gat	gat	att	tcc	335
Ala	Val	Tyr	Tyr	Cys 100	Ala	Ala	Arg	Pro	Val 105	Arg	Val	Asp	Asp	Ile 110	Ser	
ctg	ccg	gtt	ggg	ttt	gac	tac	tgg	ggc	cag	ggg	acc	cag	gtc	acc	gtc	383
Leu	Pro	Val	Gly 115	Phe	Asp	Tyr	Trp	Gly 120	Gln	Gly	Thr	Gln	Val 125	Thr	Val	
tcc	tca	gaa	ccc	aag	aca	cca	aaa	cca	caa	cca	caa	cca	caa	cca	caa	431
Ser	Ser	Glu 130	Pro	Lys	Thr	Pro	Lys 135	Pro	Gln	Pro	Gln	Pro 140	Gln	Pro	Gln	
cca	caa	ccc	aat	cct	aca	aca	gaa	tcc	aag	tgt	ccc	aaa	tgt	cca	gcc	479
Pro	Gln 145	Pro	Asn	Pro	Thr	Thr 150	Glu	Ser	Lys	Cys	Pro 155	Lys	Cys	Pro	Ala	
cct	gag	ctc	ctg	gga	ggg	ccc	tca	gtc	ttc	atc	ttc	ccc	ccg	aaa	ccc	527
Pro 160	Glu	Leu	Leu	Gly 165	Gly	Pro	Ser	Val	Phe	Ile 170	Phe	Pro	Pro	Lys	Pro 175	
aag	gac	gtc	ctc	tcc	att	tct	ggg	agg	ccc	gag	gtc	acg	tgc	gtt	gtg	575
Lys	Asp	Val	Leu	Ser 180	Ile	Ser	Gly	Arg	Pro 185	Glu	Val	Thr	Cys	Val 190	Val	
gta	gac	gtg	ggc	cag	gaa	gac	ccc	gag	gtc	agt	ttc	aac	tgg	tac	att	623
Val	Asp	Val	Gly 195	Gln	Glu	Asp	Pro	Glu 200	Val	Ser	Phe	Asn 205	Trp	Tyr	Ile	
gat	ggc	gca	gag	gtg	cga	acg	gcc	aac	acg	agg	cca	aaa	gag	gaa	cag	671
Asp	Gly 210	Ala	Glu	Val	Arg	Thr	Ala 215	Asn	Thr	Arg	Pro 220	Lys	Glu	Glu	Gln	
ttc	aac	agc	acg	tac	cgc	gtg	gtc	agc	gtc	ctg	ccc	atc	cag	cac	cag	719
Phe 225	Asn	Ser	Thr	Tyr	Arg 230	Val	Val	Ser	Val	Leu 235	Pro	Ile	Gln	His	Gln	
gac	tgg	ctg	acg	ggg	aaa	gag	ttc	aaa	tgc	aag	gtc	aac	aac	aaa	gct	767
Asp 240	Trp	Leu	Thr	Gly 245	Lys	Glu	Phe	Lys	Cys	Lys 250	Val	Asn	Asn	Lys	Ala 255	
ctc	ccg	gcc	ccc	atc	gag	aag	acc	atc	tcc	aag	gcc	aaa	ggg	cag	acc	815
Leu	Pro	Ala	Pro	Ile 260	Glu	Lys	Thr	Ile 265	Ser	Lys	Ala	Lys	Gly 270	Gln	Thr	
cgg	gag	ccg	cag	gtg	tac	gcc	ctg	gcc	cca	cac	cgg	gaa	gag	ctg	gcc	863
Arg	Glu	Pro	Gln 275	Val	Tyr	Ala	Leu 280	Ala	Pro	His	Arg	Glu 285	Glu	Leu	Ala	
aag	gac	acc	gtg	agc	gta	acc	tgc	ctg	gtc	aaa	ggc	ttc	tac	cca	cct	911
Lys	Asp	Thr 290	Val	Ser	Val	Thr 295	Cys	Leu	Val	Lys	Gly 300	Phe	Tyr	Pro	Pro	

gat	atc	aac	gtt	gag	tgg	cag	agg	aac	ggt	cag	ccg	gag	tca	gag	ggc	959
Asp	Ile	Asn	Val	Glu	Trp	Gln	Arg	Asn	Gly	Gln	Pro	Glu	Ser	Glu	Gly	
	305					310					315					
acc	tac	gcc	acc	acg	cca	ccc	cag	ctg	gac	aac	gac	ggg	acc	tac	ttc	1007
Thr	Tyr	Ala	Thr	Thr	Pro	Pro	Gln	Leu	Asp	Asn	Asp	Gly	Thr	Tyr	Phe	
	320				325					330					335	
ctc	tac	agc	aag	ctc	tcg	gtg	gga	aag	aac	acg	tgg	cag	cgg	gga	gaa	1055
Leu	Tyr	Ser	Lys	Leu	Ser	Val	Gly	Lys	Asn	Thr	Trp	Gln	Arg	Gly	Glu	
				340					345					350		
acc	ttc	acc	tgt	gtg	gtg	atg	cac	gag	gcc	ctg	cac	aac	cac	tac	acc	1103
Thr	Phe	Thr	Cys	Val	Val	Met	His	Glu	Ala	Leu	His	Asn	His	Tyr	Thr	
			355					360					365			
cag	aaa	tcc	atc	acc	cag	tct	tcg	ggt	aaa	taa	taagaattcg	agctcgaa				1154
Gln	Lys	Ser	Ile	Thr	Gln	Ser	Ser	Gly	Lys							
		370					375									

<210> 55

<211> 377

<212> PRT

<213> Artificial Sequence

 $\langle 220 \rangle$

<223> HCV33-hinge-CH2-CH3

<400> 55

Met Glu Val Gln Leu Gln Glu Ser Gly Gly Gly Leu Val Gln Ala Gly
1 5 10 15

Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Arg Ala Thr Ser Gly
20 25 30

His Gly His Tyr Gly Met Gly Trp Phe Arg Gln Val Pro Gly Lys Glu
35 40 45

Arg Glu Phe Val Ala Ala Ile Arg Trp Ser Gly Lys Glu Thr Trp Tyr
50 55 60

Lys Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys
65 70 75 80

Thr Thr Val Tyr Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala
85 90 95

Val Tyr Tyr Cys Ala Ala Arg Pro Val Arg Val Asp Asp Ile Ser Leu
100 105 110

Pro Val Gly Phe Asp Tyr Trp Gly Gln Gly Thr Gln Val Thr Val Ser
115 120 125

Ser Glu Pro Lys Thr Pro Lys Pro Gln Pro Gln Pro Gln Pro Gln Pro
 130 135 140
 Gln Pro Asn Pro Thr Thr Glu Ser Lys Cys Pro Lys Cys Pro Ala Pro
 145 150 155 160
 Glu Leu Leu Gly Gly Pro Ser Val Phe Ile Phe Pro Pro Lys Pro Lys
 165 170 175
 Asp Val Leu Ser Ile Ser Gly Arg Pro Glu Val Thr Cys Val Val Val
 180 185 190
 Asp Val Gly Gln Glu Asp Pro Glu Val Ser Phe Asn Trp Tyr Ile Asp
 195 200 205
 Gly Ala Glu Val Arg Thr Ala Asn Thr Arg Pro Lys Glu Glu Gln Phe
 210 215 220
 Asn Ser Thr Tyr Arg Val Val Ser Val Leu Pro Ile Gln His Gln Asp
 225 230 235 240
 Trp Leu Thr Gly Lys Glu Phe Lys Cys Lys Val Asn Asn Lys Ala Leu
 245 250 255
 Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Thr Arg
 260 265 270
 Glu Pro Gln Val Tyr Ala Leu Ala Pro His Arg Glu Glu Leu Ala Lys
 275 280 285
 Asp Thr Val Ser Val Thr Cys Leu Val Lys Gly Phe Tyr Pro Pro Asp
 290 295 300
 Ile Asn Val Glu Trp Gln Arg Asn Gly Gln Pro Glu Ser Glu Gly Thr
 305 310 315 320
 Tyr Ala Thr Thr Pro Pro Gln Leu Asp Asn Asp Gly Thr Tyr Phe Leu
 325 330 335
 Tyr Ser Lys Leu Ser Val Gly Lys Asn Thr Trp Gln Arg Gly Glu Thr
 340 345 350
 Phe Thr Cys Val Val Met His Glu Ala Leu His Asn His Tyr Thr Gln
 355 360 365
 Lys Ser Ile Thr Gln Ser Ser Gly Lys
 370 375

<210> 56

<211> 1172

<212> DNA

<213> Artificial Sequence

<220>

<223> hinge-HCV33-CH2-CH3-SEKDEL

<220>

<221> CDS

<222> (3)..(1154)

<400> 56

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1 5 10 15	
ggg ggc tct ctg aga ctc tcc tgt gca gcc tcg gga cgc gcc acc agt	95
Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Arg Ala Thr Ser	
20 25 30	
ggt cat ggt cac tat ggt atg ggc tgg ttc cgc cag gtt cca ggg aag	143
Gly His Gly His Tyr Gly Met Gly Trp Phe Arg Gln Val Pro Gly Lys	
35 40 45	
gag cgt gag ttt gtc gca gct att agg tgg agt ggt aaa gag aca tgg	191
Glu Arg Glu Phe Val Ala Ala Ile Arg Trp Ser Gly Lys Glu Thr Trp	
50 55 60	
tat aaa gac tcc gtg aag ggc cga ttc acc atc tcc aga gat aac gcc	239
Tyr Lys Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala	
65 70 75	
aag act acg gtt tat ctg caa atg aac agc ctg aaa cct gaa gat acg	287
Lys Thr Thr Val Tyr Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr	
80 85 90 95	
gcc gtt tat tat tgt gcc gct cga ccg gtc cgc gtg gat gat att tcc	335
Ala Val Tyr Tyr Cys Ala Ala Arg Pro Val Arg Val Asp Asp Ile Ser	
100 105 110	
ctg ccg gtt ggg ttt gac tac tgg ggc cag ggg acc cag gtc acc gtc	383
Leu Pro Val Gly Phe Asp Tyr Trp Gly Gln Gly Thr Gln Val Thr Val	
115 120 125	
tcc tca gaa ccc aag aca cca aaa cca caa cca caa cca caa cca	431
Ser Ser Glu Pro Lys Thr Pro Lys Pro Gln Pro Gln Pro Gln Pro Gln	
130 135 140	
cca caa ccc aat cct aca aca gaa tcc aag tgt ccc aaa tgt cca gcc	479
Pro Gln Pro Asn Pro Thr Thr Glu Ser Lys Cys Pro Lys Cys Pro Ala	
145 150 155	
cct gag ctc ctg gga ggg ccc tca gtc ttc atc ttc ccc ccg aaa ccc	527
Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Ile Phe Pro Pro Lys Pro	
160 165 170 175	
aag gac gtc ctc tcc att tct ggg agg ccc gag gtc acg tgc gtt gtg	575
Lys Asp Val Leu Ser Ile Ser Gly Arg Pro Glu Val Thr Cys Val Val	
180 185 190	
gta gac gtg ggc cag gaa gac ccc gag gtc agt ttc aac tgg tac att	623
Val Asp Val Gly Gln Glu Asp Pro Glu Val Ser Phe Asn Trp Tyr Ile	
195 200 205	

[illegible]

<210> 57

<212> PRT

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Met 1	Glu	Val	Gln	Leu 5	Gln	Glu	Ser	Gly	Gly 10	Gly	Leu	Val	Gln	Ala 15	Gly
Gly	Ser	Leu	Arg 20	Leu	Ser	Cys	Ala	Ala 25	Ser	Gly	Arg	Ala	Thr 30	Ser	Gly
His	Gly	His 35	Tyr	Gly	Met	Gly	Trp 40	Phe	Arg	Gln	Val	Pro 45	Gly	Lys	Glu
Arg	Glu 50	Phe	Val	Ala	Ala	Ile 55	Arg	Trp	Ser	Gly	Lys 60	Glu	Thr	Trp	Tyr
Lys 65	Asp	Ser	Val	Lys	Gly 70	Arg	Phe	Thr	Ile	Ser 75	Arg	Asp	Asn	Ala	Lys 80
Thr	Thr	Val	Tyr	Leu 85	Gln	Met	Asn	Ser	Leu 90	Lys	Pro	Glu	Asp	Thr 95	Ala
Val	Tyr	Tyr	Cys 100	Ala	Ala	Arg	Pro	Val 105	Arg	Val	Asp	Asp	Ile 110	Ser	Leu
Pro	Val	Gly 115	Phe	Asp	Tyr	Trp	Gly 120	Gln	Gly	Thr	Gln	Val 125	Thr	Val	Ser
Ser	Glu 130	Pro	Lys	Thr	Pro	Lys 135	Pro	Gln	Pro	Gln	Pro 140	Gln	Pro	Gln	Pro
Gln 145	Pro	Asn	Pro	Thr	Thr 150	Glu	Ser	Lys	Cys	Pro 155	Lys	Cys	Pro	Ala	Pro 160
Glu	Leu	Leu	Gly	Gly 165	Pro	Ser	Val	Phe	Ile 170	Phe	Pro	Pro	Lys	Pro 175	Lys
Asp	Val	Leu	Ser 180	Ile	Ser	Gly	Arg	Pro 185	Glu	Val	Thr	Cys	Val 190	Val	Val
Asp	Val	Gly 195	Gln	Glu	Asp	Pro	Glu 200	Val	Ser	Phe	Asn	Trp 205	Tyr	Ile	Asp
Gly	Ala 210	Glu	Val	Arg	Thr	Ala 215	Asn	Thr	Arg	Pro	Lys 220	Glu	Glu	Gln	Phe
Asn 225	Ser	Thr	Tyr	Arg	Val 230	Val	Ser	Val	Leu	Pro 235	Ile	Gln	His	Gln	Asp 240
Trp	Leu	Thr	Gly	Lys 245	Glu	Phe	Lys	Cys	Lys 250	Val	Asn	Asn	Lys	Ala 255	Leu
Pro	Ala	Pro	Ile 260	Glu	Lys	Thr	Ile	Ser 265	Lys	Ala	Lys	Gly	Gln 270	Thr	Arg
Glu	Pro	Gln 275	Val	Tyr	Ala	Leu	Ala 280	Pro	His	Arg	Glu	Glu 285	Leu	Ala	Lys
Asp	Thr 290	Val	Ser	Val	Thr	Cys 295	Leu	Val	Lys	Gly	Phe 300	Tyr	Pro	Pro	Asp
Ile 305	Asn	Val	Glu	Trp	Gln 310	Arg	Asn	Gly	Gln	Pro 315	Glu	Ser	Glu	Gly	Thr 320

Tyr Ala Thr Thr Pro Pro Gln Leu Asp Asn Asp Gly Thr Tyr Phe Leu
 325 330 335
 Tyr Ser Lys Leu Ser Val Gly Lys Asn Thr Trp Gln Arg Gly Glu Thr
 340 345 350
 Phe Thr Cys Val Val Met His Glu Ala Leu His Asn His Tyr Thr Gln
 355 360 365
 Lys Ser Ile Thr Gln Ser Ser Gly Lys Ser Glu Lys Asp Glu Leu
 370 375 380

<210> 58

<211> 31

<212> DNA

<213> Artificial Sequence

<220>

<223> PCR primer

<400> 58

aggggaccca ggtaaccgtc tcctcagaac c

31

<210> 59

<211> 37

<212> DNA

<213> Artificial Sequence

<220>

<223> PCR primer

<400> 59

gagctttgtt gttgaccttg catttgaact ctttccc

37

<210> 60

<211> 26

<212> DNA

<213> Artificial Sequence

<220>

<223> PCR primer

<400> 60

caaatgcaag gtcaacaaca aagctc

26

<210> 61

<211> 42

<212> DNA

<213> Artificial Sequence

<220>

<223> PCR primer

<400> 61

ttcgagctcg aattcttatt atttaccga agactgggtg at

42

<210> 62

<211> 27

<212> DNA

<213> Artificial Sequence

<220>

<223> sequencing primer

<400> 62

ctgaggagac ggtgacctgg gtcccct

27

<210> 63

<211> 46

<212> DNA

<213> Artificial Sequence

<220>

<223> PCR primer

<400> 63

agccccctgag ctctgggag ggcctcagt cttcatcttc cccccg

46

<210> 64

<211> 61

<212> DNA

<213> Artificial Sequence

094260

<220>

<223> PCR primer

<400> 64

ttcgagctcg aattcttatt atttaccgga agactgggtg atggatttct gggtagtg 60

g 61

<210> 65

<211> 79

<212> DNA

<213> Artificial Sequence

<220>

<223> PCR primer

<400> 65

ttcgagctcg aattcttatt atagctcatc tttctcagat ttaccggaag actgggtgat 60

ggatttctgg gtgtagtg 79

<210> 66

<211> 461

<212> DNA

<213> Artificial Sequence

<220>

<223> VHH with linker

<220>

<221> CDS

<222> (3)..(449)

<400> 66

cc atg gcc cag gtg cag ctg cag gag tct ggg gga ggc ttg gtg cag 47

Met Ala Gln Val Gln Leu Gln Glu Ser Gly Gly Gly Leu Val Gln
1 5 10 15

gct ggg ggg tct ctg agg ctc tcc tgt gca gcc tct gga agc att ttc 95

Ala Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Ser Ile Phe
20 25 30


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aga cgt ccg cat atg ggt tgg ttc cgc cag gct cca ggg cag gag cgc      143
Arg Arg Pro His Met Gly Trp Phe Arg Gln Ala Pro Gly Gln Glu Arg
          35                      40                      45

gag ttg gtc gca ctg att tct gcg ggt ggt cgt aca tgg tat gca gac      191
Glu Leu Val Ala Leu Ile Ser Ala Gly Gly Arg Thr Trp Tyr Ala Asp
          50                      55                      60

tcc gtg aag ggc cga ttc acc atc tcc aga gac aac gcc aag aac acg      239
Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr
          65                      70                      75

ctg tat ctg caa atg aac agc ctg aaa cct gag gac acg gcc gtt tat      287
Leu Tyr Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr
          80                      85                      90                      95

tac tgt act gcc ggg ggt tcg tac tgg ggc cag ggg acc cag gtc acc      335
Tyr Cys Thr Ala Gly Gly Ser Tyr Trp Gly Gln Gly Thr Gln Val Thr
          100                      105                      110

gtc gcc tca gaa ccc aag aca cca aaa cca caa cca gcg gcc gcc cat      383
Val Ala Ser Glu Pro Lys Thr Pro Lys Pro Gln Pro Ala Ala Ala His
          115                      120                      125

cac cat cac cat cac ggg gcc gca gaa caa aaa ctc atc tca gaa gag      431
His His His His His Gly Ala Ala Glu Gln Lys Leu Ile Ser Glu Glu
          130                      135                      140

gat ctg aat ggg gcc gca tagtaacaat tg      461
Asp Leu Asn Gly Ala Ala
          145

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<210> 67

<211> 149

<212> PRT

<213> Artificial Sequence

<220>

<223> VHH with linker

<400> 67

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Met Ala Gln Val Gln Leu Gln Glu Ser Gly Gly Gly Leu Val Gln Ala
1          5          10          15

Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Ser Ile Phe Arg
          20          25          30

Arg Pro His Met Gly Trp Phe Arg Gln Ala Pro Gly Gln Glu Arg Glu
          35          40          45

Leu Val Ala Leu Ile Ser Ala Gly Gly Arg Thr Trp Tyr Ala Asp Ser
          50          55          60

Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu
65          70          75          80

```

Tyr Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr
 85 90 95
 Cys Thr Ala Gly Gly Ser Tyr Trp Gly Gln Gly Thr Gln Val Thr Val
 100 105 110
 Ala Ser Glu Pro Lys Thr Pro Lys Pro Gln Pro Ala Ala Ala His His
 115 120 125
 His His His His Gly Ala Ala Glu Gln Lys Leu Ile Ser Glu Glu Asp
 130 135 140
 Leu Asn Gly Ala Ala
 145